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MEMORANDUM FOR US EPA
NCEA (MD-52)
RTP NC 27711
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FROM: AFRL/HEST
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SUBJECT: Consultative Letter, AFRL-HE-WP-CL-1998-0022, Pharmacokinetics of Iodide Uptake Inhibition in the Thyroid by Perchlorate

1. The Operational Toxicology Branch intends to collect pharmacokinetic data sets to develop a mechanistically motivated, biologically-based pharmacokinetic (BBPK) models to predict dose-response characteristics for perchlorate induced inhibition of uptake of iodide in the thyroid and subsequent alternations in thyroid hormones (TSH, T3, T4) in rodents and humans. Phase I of the research is currently underway. Phase I research consists of conducting ^{125}I and $^{36}ClO_4$ studies in the adult male rat, ^{125}I labeled T3 and T4 studies, and ^{125}I studies with cold ClO_4. To obtain biochemical constants for symporter uptake of iodide and perchlorate into the thyroid lumen, several doses of labeled perchlorate will be used, while the labeled iodide dose remains constant. In other studies, animals will be placed on drinking water containing perchlorate for several weeks and at specified times dosed with labeled iodide to obtained information on the relationship between sustained systemic concentrations of perchlorate and inhibition of iodide uptake into the thyroid lumen. Perchlorate concentrations in drinking water will be consistent with recent perchlorate toxicity tests (calculated doses of 0.01 to 30 mg/kg). Animals will be dosed with labeled hormones (T3, T4) to provide pharmacokinetic information on the hormones, such as protein-bound and free half-life values for T3 and T4 in blood, and rates of deiodinase activity. Phase II of the research is more substantial and will require funding and extramural research support. Three tasks are identified:

- Obtain pharmacokinetic information on the inhibition of iodide uptake by perchlorate in both the pregnant rat and its fetus and the lactating rat and its nursing neonate. PBPK models will be developed to describe inhibition of iodide uptake in the thyroid by modifying previously published pregnancy and lactation models in the rat (Fisher et al., 1989, Fisher et al., 1990).
  - Rodent PBPK models for describing perchlorate induced inhibition of iodide uptake into the thyroid lumen from the blood will be expanded for the adult male rat (Phase I), pregnant rat and fetus and the lactating rat and nursing pup (Phase II) to include thyroid hormones (TSH, T3 and T4).

- Human dose-response pharmacokinetic experiments will be performed to quantify the degree of inhibition of iodide uptake in thyroid by perchlorate and the resulting alternations in thyroid hormone homeostasis. A human mechanistically motivated BBPK model will be constructed for perchlorate induced inhibition of uptake the iodide and alternations in thyroid hormone homeostasis.

1. The payoff for this research effort is in *the elimination of the interspecies uncertainty factor* with the development and validation of biologically motivated mechanistic models for predicting perchlorate induced thyroid perturbations in rodents and humans.

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Attachments
1. Mathematical Modeling Considerations for Iodide and Perchlorate
2. Schematic and Figure Legend

Mathematical Modeling Considerations for Iodide and Perchlorate (attachment 1)

Several years ago J. Wolf (1964, Transport of Iodide and Other Anions in the Thyroid Gland, *Physiology Review*, pp. 45-90) reviewed the mathematical models developed by Wollman and colleagues for predicting iodide uptake in the thyroid. These models are classical compartmental models describing the thyroid gland with 1 to 3 compartments and the body (blood) as one compartment. Clearance rates (rate of clearance of iodide from blood) were used to describe the active transport of iodide by the symporter into the thyroid and 1st order binding and transfer rate constants were used to describe organification and transfer of iodide within and from the thyroid. Data and model predictions were expressed as ratios of radioactivity counts (thyroid/serum or plasma). Since this time, it appears that no modeling efforts to describe iodide uptake and inhibition by other anions have been published. However, efforts to quantitatively describe hormone formation have been undertaken (J. H. Oppenheimer and M. I. Surks, 1974, Quantitative Aspects of Hormone Production, Distribution, Metabolism, and Activity, *Handbook of Physiology- Endocrinology III, Chapter 13*, pp 197-214). Most recently Kohn et al. (1996, A Mechanistic Model of Effects of Dioxin on the Thryoid Hormones in the Rat, *Toxicol. Appl. Pharmacol.* pp. 29-48) developed an elaborate mechanistic model for thyroid hormones (not iodide) and dioxin in the rat.

Significant data gaps exist for development of mechanistically motivated BBPK models for iodide and perchlorate in the rat and human. Although experimental studies in rats and humans have been carried out with radio labeled iodide and perchlorate, much of the published data is expressed simply as thyroid/blood ratios of radioactivity counts, with little or no information given on dose (mass) or concentrations in biological tissues. Relevant data for co-administration of iodide and perchlorate is not available for model development unless the model is configured to predict only thyroid/blood ratios. Interestingly, Km values that described the affinity of both iodide (Km_{iodide}) and perchlorate (Km_{clo4}) for the carrier protein on the symporter are given in the literature, although never used in mathematical models. No values for maximum rate of uptake of iodide ($Vmax_{iodide}$) or perchlorate ($Vmax_{clo4}$) from blood into the thyroid lumen were found in the literature.

Diffusion of iodide into the thyroid lumen is probably only significant if iodide is in excess in the blood (abnormal concentrations) and/or the symporter is not functioning. If the symporter is not functioning, a diffusion gradient may develop between the thyroid and the blood, allowing iodide to diffuse into the thyroid lumen down a concentration gradient. Evidence showing the contribution of active uptake versus diffusion is clearly depicted with perchlorate by Chow and Woodbury (1970, Kinetics of Distribution of Radioactive Perchlorate in Rat and Guinea Pig Glands, J. Endocr.) (see below).

Table 1, Changes of perchlorate concentration in plasma and thyroid gland in i.p. dosed Sprague-Dawley rats

Dose	0.69 mg $K^{36}ClO_4$/kg bodyweight[a]	0.69 mg $K^{36}ClO_4$/kg bodyweight[a]	14 mg $K^{36}ClO_4$/kg bodyweight[a]	14 mg $K^{36}ClO_4$/kg bodyweight[a]	280 mg $K^{36}ClO_4$/kg bodyweight[a]	280 mg $K^{36}ClO_4$/kg bodyweight[a]
Time (hr)	Thyroid $^{36}ClO_4^-$ (mg/kg thyroid)[b]	Plasma $^{36}ClO_4^-$ (mg/L plasma)[b]	Thyroid $^{36}ClO_4^-$ (mg/kg thyroid)[b]	Plasma $^{36}ClO_4^-$ (mg/L plasma)[b]	Thyroid $^{36}ClO_4^-$ (mg/kg thyroid)[b]	Plasma $^{36}ClO_4^-$ (mg/L plasma)[b]
0.033	8.1	1.2	14	27	87	333
0.067	13	1.6	16	42	121	530
0.13	16	2.1	27	46	159	601
0.25	16	2.5	32	46	244	670
0.50	17	1.8	34	39	228	637
1.0	17	1.8	33	42	234	584
2.0	17	1.6	37	40	232	589
4.0	18	1.3	40	43	270	613

[a] 138.5 g/mol $KClO_4$

[b] assume 0.226 kg bodyweight; actual weight 226 ± 4 g

These authors demonstrated that perchlorate is actively sequestered by the thyroid gland with a low dose (0.69 mg/kg) of perchlorate. The capacity of the symporter to actively sequester perchlorate is exceeded at higher doses of perchlorate (14 and 280 mg/kg), resulting in diffusion as the predominant process for uptake of perchlorate into the thyroid. These data suggest that maximal inhibition of active uptake of iodide in the thyroid by perchlorate probably occurs below 14 mg/kg . If perchlorate induced inhibition of active uptake of iodide is substantial, iodide may still enter the thyroid by diffusion, albeit a lesser amount. If inhibition of active uptake of iodide in the thyroid by perchlorate is incomplete, then iodide may still be actively sequestered into the thyroid, albeit a lesser amount. The Chow and Woodbury study suggests that the maximal effect of perchlorate on active uptake of iodide is probably less than 14 mg/kg and above 0.69 mg/kg. Thus perchlorate induced thyroid hormone perturbations may plateau in adult rats dosed with perchlorate greater than around 5-10 mg/kg of perchlorate.

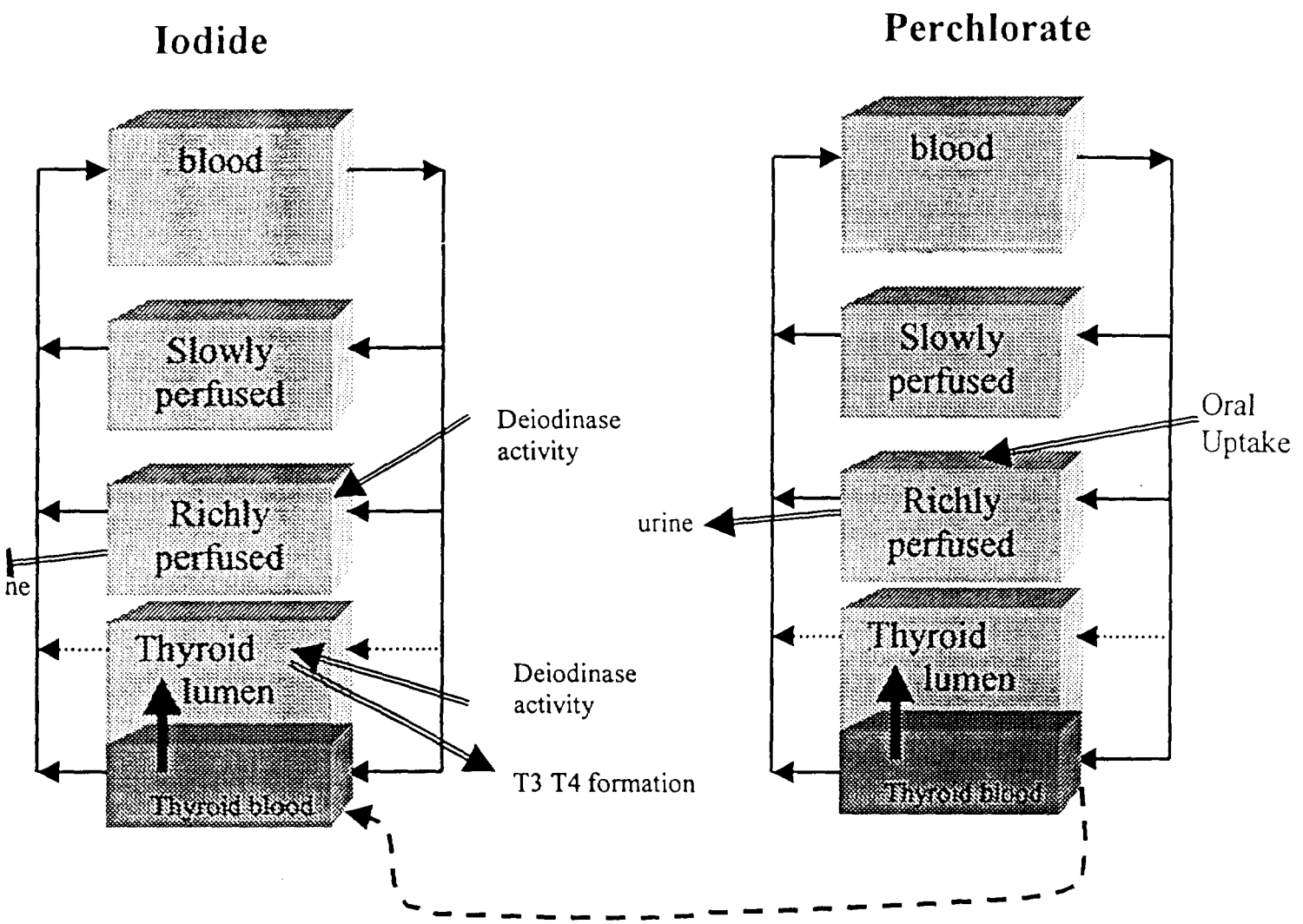
STATUS OF RESEARCH

The first phase of model development will focus on conducting laboratory studies in the adult male rats. Data collected from these kinetic studies with ^{125}I, cold and radiolabeled perchlorate

($^{36}ClO_4$) will be used to construct a model to predict the degree of inhibition for uptake of iodide by the symporter in the thyroid as a function of systemic concentration of perchlorate under single and repeated perchlorate dosing regimes. Two animal use protocols (and amendments) are currently being used. Other amendments are anticipated (eg., dosing with labeled T_4 and T_3 to quantify deiodinase activity).

Two PK studies with radiolabeled iodide (2.6 pg/kg and 33 ug/kg) have been conducted. Further studies are anticipated to fill in gaps. Perchlorate studies will begin in October, 1998. Iodide and perchlorate models are under development and a tentative schematic of the models are provided with a figure legend.

Attachment 2. Tentative schematic of kinetic portion of biologically based pharmacokinetic model for iodide and perchlorate in the rat and Figure legend explaining components of the model.



Model predicted concentration of perchlorate in blood which inhibits uptake of iodide in thyroid lumen

FIGURE LEGEND FOR MODEL SCHEMATIC

Schematic of the kinetic aspects of a tentative iodide/perchlorate biologically based pharmacokinetic model for the adult rat. Four-compartments (boxes entitled slowly and rapidly perfused, blood and thyroid compartments) are used to describe the kinetics of the iodide and perchlorate ions. The thyroid compartment is composed of blood and lumen sub-compartments. The blood compartment in the thyroid gland allows for active uptake of iodide from the thyroid blood into the thyroid lumen. If required, passive uptake (diffusion, dotted arrow) of iodide into the thyroid lumen will be described by a venous equilibration term. Other compartments in the model are described with venous equilibration equations. The iodide and perchlorate models are linked by equations that describe the competitive inhibition of active uptake of both iodide and perchlorate into the thyroid lumen by the symporter.

The iodide model will account for several key biological determinants responsible for describing the kinetics of iodide. The key biological determinants are 1) passive and saturable uptake of iodide from the thyroid blood into the thyroid lumen, 2) distribution of iodide into slowly and rapidly perfused compartments and blood compartment, 3) deiodinase activity (T_4 and $T_3 \rightarrow$ iodide) in thyroid lumen and richly perfused tissue compartments, 4) binding (oxidation and organification) of iodide in thyroid lumen, 5) diffusion of iodide from thyroid lumen to blood, 6) loss of bound (organified) iodide from thyroid lumen to the body and 7) urinary excretion of iodide. These features in the model are represented as arrows in the schematic. Dietary uptake of iodine will be added to the model at a later date.

The main features of the perchlorate model will include oral uptake of perchlorate, distribution into blood, richly and slowly perfused compartments, passive and saturable uptake into thyroid by symporter and urinary excretion of perchlorate.

To further clarify how the iodide and perchlorate model are linked by a competitive inhibition term, the equation for inhibition of active uptake of iodide by perchlorate is presented, where,

$$dA_{iodide}/dt = Vmax_{iodide} \times C_{iodide} / Km_{iodide} \times (1.0 + C_{clo4} / Km_{clo4}) + C_{iodide}$$

$Vmax_{iodid}$= maximal uptake of iodide (fg/hr) at symporter
Km_{iodide} = Michaelis-Menton affinity constant (fg/L) for iodide
Km_{clo4} = Michaelis-Menton affinity constant (mg/L) for perchlorate
C_{iodide} = Concentration of iodide in blood perfusing thyroid (fg/L)
C_{clo4} = Concentration of perchlorate in blood perfusing thyroid (mg/L)

The key variables that describe inhibition of iodide uptake by perchlorate are Km_{clo4} for perchlorate [at the protein binding site of the symporter] and the model predicted blood concentrations of perchlorate (C_{clo4}) in thyroid blood. The key variables that describe uptake of iodide into the thyroid by the symporter are $Vmax_{iodide}$ and Km_{iodide} and the blood concentration of iodide in thyroid blood (C_{iodide}). In a similar fashion the influence of iodide on perchlorate uptake into thyroid lumen will be described.